



Applicants : P. CASARA
A-M. CHOLLET
A. DHAINAUT
L. BERT
P. LESTAGE
B. LOCKHART

Serial N°: 10/589,831

Filed : August 17, 2006

Title : Azabicyclic compounds, a process for their preparation and
pharmaceutical compositions containing them

Art Unit : 1626

Examiner : REI-TSANG SHIAO

Honorable Commissioner of Patents and Trademarks

Washington, D.C. 20231

DECLARATION UNDER 37 CFR 1.132

I, Pierre LESTAGE, a citizen of France, 9, Allée de la Grande Terre, 78170 La Celle St Cloud, France, declare and say that :

I hold the degree of Doctor in Philosophy in Neurosciences from the University of Lyon I, France, in 1987.

Since November 1987 I have been working in SERVIER :

From November 1987 to December 1994 : I had a Senior Research position and I was responsible for the development of *in vivo* behavioral models as part of a screening program for the discovery of novel cognition enhancers and/or neuroprotective drugs towards acute and chronic neurodegenerative diseases.

- From January 1995 to September 1998 : I was Research Project Leader in the field of neuropharmacology. I was responsible for various research projects aimed to the discovery of novel cognition enhancers and/or neuroprotective drugs.
- From October 1998 to December 1999 : I was director of the department of Neurobiology at Servier, responsible for all the *in vivo* preclinical experiments performed in the field of neuropharmacology for the discovery of novel cognition enhancers and neuroprotective drugs.
- From January 2000-present : I am Director of the Division of Cerebral Pathology at Servier. The main topic of research of this division is the discovery of novel cognition enhancers and neuroprotective drugs against pathological cerebral aging, acute and chronic neurodegenerative diseases.

I am the author or co-author of 49 patents and 45 international publications, the majority of which are devoted to Neuropharmacology.

I am one of the co-inventors of US Patent Application Serial n° 10/589,831 filed August 17, 2006 concerning " azabicyclic compounds, a process for their preparation and pharmaceutical compositions containing them ".

I am thoroughly familiar with the above-mentioned patent application and fully support the pharmacological experiments contained therein which were performed either by me or under my supervision. I also fully support the conclusions derived and the arguments presented as concerns the therapeutic interest of the compounds described.

Disorders of learning and memory are characteristic of cerebral aging. As a greater proportion of the population survives beyond the age of 75, an increasing number of individuals will likely suffer from age-related cognitive and attentional deficits, and consequently a reduced quality of life. Furthermore, the increasing proportion of elderly in the population is accompanied by an increase of chronic age-related cerebral neurodegenerative diseases (Alzheimer's and Parkinson's diseases; in

particular) characterized by memory impairment and cognitive dysfunction. Age-related cognitive disorders are due to a lower efficacy of the neurons to both synthesize and release certain neurotransmitters. Furthermore, in an aging brain often exposed to a reduced threshold-stimulation, there is a gradual loss of synaptic plasticity and of neuronal processes. In the case of neurodegenerative diseases, this neuronal loss is accelerated in specific brain regions. Under these conditions, cognitive functions decline as they cannot be maintained by the remaining and deficient neuronal networks. Among the various neurotransmitters, central histamine via H3 receptors plays a major role in the control of arousal and cognitive functions (Witkin and Nelson, 2004). Cognition is not a unitary phenomenon as it is a complex of multiple integrated neurological and behavioural activities of which arousal has ample documentation as a key player. Therefore, it has been argued that a compound that positively modulates attention and vigilance, as it has been reported for H3 antagonists, would be valuable in correcting cognitive deficiencies for which these functions were reduced (Leurs *et al.*, 1998; Katz, 2004.).

Histamine interacts with 4 types of receptors, which differ in distribution, pharmacology, and function. The histamine H3 receptor is a member of the large superfamily of G protein-coupled receptors (GPCRs) that are characterized by the presence of seven putative transmembrane-spanning domains. The third histamine receptor subtype (H3) was identified by Arrang *et al.*, 1983, and it expressed with the highest abundance in the central nervous system. The H3 receptor has generally pre-synaptic localization and inhibits the synthesis and release of histamine and other neurotransmitters, like acetylcholine, serotonin, noradrenaline, and dopamine. Its involvement in different brain functions, like learning processes, arousal and regulation of food intake, has led to the proposal of H3 antagonists for different therapeutic applications, especially in the treatment of Alzheimer's disease, attention deficit hyperactivity disorder (ADHD) and obesity. Consequently, a wide range of structurally diverse ligands has been synthesized (see Leurs *et al.*, 2005 for a general overview) as pharmacological tools or as potential drug candidates.

Data exist, that challenge the idea that H3 receptor antagonists may be effective in disorders involving cognition. Although H3 antagonists/inverse agonists impair contextual fear conditioning (Passani *et al.*, 2001), their administration produces

significant enhancement in several cognitive tasks. Pharmacological blockade of H3 receptors in the adult rat significantly improves social memory (Prast *et al.*, 1996), enhances attention as evaluated in the five-choice, serial-reaction time test (Ligneau *et al.*, 1998), and improves cognitive performance in the five-trial inhibitory avoidance task (Fox *et al.*, 2002, 2003). Procognitive effects of H3 receptor antagonists/inverse agonists have been observed also in cognitively impaired animals. For example, thioperamide significantly improves the response latency in a passive avoidance response in senescence-accelerated mice, characterized by a marked age-accelerated deterioration in learning tasks (Meguro *et al.*, 1995). Consistently, two H3 receptor antagonists/inverse agonists, thioperamide, and clobenpropit, fully reverted scopolamine-elicited impairments in a passive avoidance response and object-recognition test in the rat (Giovannini *et al.*, 1999). Similarly, FUB 181, an H3 receptor antagonist (Stark *et al.*, 1998), significantly ameliorates performances of scopolamine-impaired mice in the elevated plus-maze test (Onodera *et al.*, 1988). The memory enhancing effects of H3 antagonists might be useful in the treatment of Alzheimer's disease, especially because histaminergic neurons seem to be largely spared in this neurodegenerative disease, and also because H3 antagonists facilitate cholinergic neurotransmission which is highly deficient in Alzheimer's disease (Bacciottini *et al.*, 2001).

Under these circumstances, the compounds of the present art were synthesized as a novel H3 antagonists.

1- NEUROCHEMICAL EFFECTS ON THE COMPOUNDS OF THE PRESENT INVENTION

1.1 Effect on extracellular levels of histamine in the prefrontal cortex of freely moving rats

Male albino rats of a Wistar derived strain (280-350 g; Harlan, Zeist, The Netherlands) were used for the experiments. Experiments were concordant with the declarations of Helsinki and were approved by the animal Care committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

Rats were anaesthetised and homemade I-shaped probes (dialyzable surface 4 mm) were inserted in prefrontal cortex (PFC). Experiments were performed 24-48 hours

after surgery. At the day of the experiment, animals were connected with flexible PEEK tubing to a microperfusion pump and perfused with a CSF, containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, and 1.2 mM MgCl₂ at a flow rate of 1.5 µl/min. 20 minute microdialysis samples were collected online in HPLC loops and injected automatically onto the column. Compounds were injected in 1% tween 80 in saline (1 ml/kg). Four consecutive microdialysis samples with less than 40 % variation were taken as baseline levels and set at 100 %. Drug effects were expressed as percentages of basal level (mean + SEM). Drug effects are compared to vehicle administration using two way ANOVA for repeated measures, followed by Student Newman Keul's post-hoc analysis. Level of significance was set at $p < 0.05$.

➤ Results indicated that Example 22 of the present invention administered IP acutely dose-dependently increased (1mg/kg : + 200%, 3 mg/kg : + 400% and 10 mg/kg : + 750%) extracellular levels of histamine in the prefrontal cortex of freely moving rats.

1.2 Effect on extracellular levels of acetylcholine (ACh) in the prefrontal cortex of freely moving rats

Male Wistar rats (280-320 g) were stereotaxically implanted with a guide-cannula in the prefrontal cortex. Six or seven days later, the microdialysis probe (CMA12, Ø 0.5 mm, length 4 mm, Carnegie Medicin, Phymep, France) was lowered in the guide-cannula and was continuously perfused at a flow rate of 1 µl/min with an artificial cerebro-spinal fluid supplemented with 20 nM neostigmine. After a two hour stabilisation period, compounds (1-10 mg/kg i.p.) were administered and dialysates were collected every 30 min. Extracerebral ACh was assayed by HPLC coupled to electrochemical detection.

➤ Example 22 significantly increased acetylcholine levels in the prefrontal cortex of freely-moving, at the dose of 10 mg/kg IP (+ 300%).

1.3 *Effect on extracellular levels of monoamines (noradrenaline, dopamine and serotonin) in the prefrontal cortex of freely moving rat*

Male albino rats of a Wistar derived strain (280-350 g; Harlan, Zeist, The Netherlands) were used for the experiments. Experiments were concordant with the declarations of Helsinki and were approved by the animal Care committee of the Faculty of Mathematics and Natural Sciences of the University of Groningen.

Rats were anaesthetised and homemade I-shaped probes (dialyzable surface 4 mm) were inserted in prefrontal cortex (PFC). Experiments were performed 24-48 hours after surgery. At the day of the experiment, animals were connected with flexible PEEK tubing to a microperfusion pump and perfused with a CSF, containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, and 1.2 mM MgCl₂ at a flow rate of 1.5 µl/min. 30 minute microdialysis samples were collected online in HPLC loops and injected automatically onto the column. Compounds were injected in 1% tween 80 in saline (1 ml/kg). Four consecutive microdialysis samples with less than 40 % variation were taken as baseline levels and set at 100 %. Drug effects were compared to vehicle administration using two way ANOVA for repeated measures, followed by Student Newman Keul's post-hoc analysis. Level of significance was set at $p < 0.05$.

> The highest dose of Example 22 (10 mg/kg, IP) elicited a significant increase of norepinephrine (+180%), dopamine (+216%) and serotonin (+ 230%) levels in the prefrontal cortex of freely moving rats.

As a whole, the neurochemical effects of the compounds of the present invention are in favour of therapeutic effects of these compounds in situation in which dysfunction of the major neurochemical pathways occurs in the brain in particular in case of cholinergic, histaminergic and monoaminergic hypofunction. That is mainly in diseases in which cognition and/or mood disorders occur in particular in chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, vascular dementia and mood disorders such as anxiety. To confirm these effects a set of behavioural experiments have been performed.

2- BEHAVIORAL EXPERIMENTS

The following studies were conducted in order to evaluate the capacity of compounds of the present invention to improve cognitive functions which are impaired in neurodegenerative diseases and in mood disorders.

2.1. *Effect on models of episodic-like memory*

2.1.1 *Social recognition test in the Wistar Rat*

Adult Wistar rats (400-420 g, CERJ) were submitted to a social recognition test. Each rat was exposed to a juvenile rat in two trials (5 min each) separated by an inter trial interval of two hours. The time (sec) spent in investigating the juvenile was recorded, a decrease of investigation on the second trial indicating that the rat recognized the juvenile. In control rats, the times of investigation were the same for the two trials demonstrating that the animals no longer recognized the juvenile rat in the second trial. Statistical analyses were performed on the difference in investigation time between the two trials.

> Results indicate that Example 22 of the present invention improved social recognition memory at active doses of 0.3, 1 and 3 mg/kg i.p.

2.1.2 *Object recognition test in the Sprague Dawley rat*

The one-trial object recognition paradigm measures a form of episodic memory in the rat (Ennaceur and Delacour, 1988). Recognition is measured by the time spent by rats in exploring two different objects, one familiar and the other new. With an inter-trial interval of 2 hours, normal rats spend more time exploring the new object which demonstrates that they recognize the familiar one. After a retention interval of 24 h, the rats do not discriminate between the two objects as indicated by similar times spent in exploring them. Animals (220-330g; CERJ) were submitted to three sessions of the test. The first one was a session of habituation (3 min) to the experimental conditions. During the acquisition session, the rats were presented with the two similar objects and were removed as soon as they reached 15 s of objects exploration with a cut-off time of 4 min. The third session (3 min) consisted of the recognition test between the familiar and the new object. Statistical analyses were

performed both on individual exploration of familiar and new objects and on the difference in duration of exploration of the two objects.

- Effects of compounds on scopolamine-induced amnesia

Rats were treated with vehicle or compounds (0.3-1-3 mg/kg) by oral route and with saline 0.9% or scopolamine (0.3 mg/kg IP), respectively 90 min and 30 min before the acquisition trial. The recognition test was performed 2 hours later.

➤ Results indicated that scopolamine (0.3 mg/kg IP) administered 30 min before acquisition induced a significant learning deficit (amnesia) compared with vehicle-treated rats. Oral administration of Example 22 (0.3-1-3 mg/kg), 60 min before scopolamine increased in a dose dependent manner and significantly at 3 mg/kg the recognition of the familiar object.

- Effect of compounds on natural forgetting: consolidation process

Compounds (0.3-1-3 mg/kg) were administered by oral route after the acquisition session. The recognition test was performed 24 hours later.

➤ Results indicated that Example 22 increased significantly the recognition of the familiar object at the doses of 0.3 and 1 mg/kg compared to control group been active on consolidation process which is impaired in Alzheimer's disease for example.

2.2 Effect of compounds on models of spatial working memory

2.2.1 Spontaneous alternation test in the C57BL/6 mice

Spontaneous alternation is the innate tendency of rodents to alternate free choices in a T-maze over a series of successive runs (Dember and Fowler, 1958). Spontaneous alternation is defined as a visit to the other of the two goals arms a T-maze than that visited in the previous trial. This sequential procedure relies working memory since the ability to alternate requires that the subject retain specific information, which varies from trial to trial. 30 minutes after drug or vehicle injections, mice (4-5 months, CERJ) were placed in a T-maze and were submitted to a session of 7 free trials separated by a 180-s inter-trial interval. The statistical analysis was performed on the

percentage of alternation over the 7 trials, used as an index of working memory performance.

➤ Results indicate that Example 22 improved spatial working memory performances in mice at active dose of 0.03 and 0.1 mg/kg IP.

2.2.2 Working memory: Delayed Matching To Sample task in aged monkeys

The experiments aimed to evaluate the effects of compounds after oral administration on a short-term visual memory task in aged rhesus monkeys. The memory task used, delayed matching-to-sample (DMTS), is one which has been shown sensitive to the age-related decline in cognitive function seen both in animals and in humans, and has been shown to be sensitive to several putative cognition enhancers.

Seven aged female rhesus monkeys, aged 17 – 32 years and weighing 8.0 ± 0.98 kg, were well trained (> 100 individual sessions) in the DMTS task. The animals were maintained on tap water (unlimited) and standard laboratory monkey chow supplemented with fruit and vegetables. The animals were maintained on a feeding schedule such that approximately 15% of their normal daily food intake (except weekends) was derived from 300 mg banana-flavoured reinforcement food pellets obtained during experimental sessions. The remainder was made available following each test session. On weekends the animals were fed twice per day. Each animal had previously participated in one or more pharmacological studies of test substances that were considered to have fully reversible actions. At least a 4-week washout period preceded the initiation of this study. The aged female monkeys used in this study were peri-menopausal, i.e. still cycling but infrequently. Testing was always carried out between menstrual periods. The monkeys were maintained on a 12 hr light-dark cycle (artificial light on at 6:30 hr) and were tested each weekday between 9.00 and 14.00 hr. Room temperature and relative humidity were maintained at $22 \pm 1^\circ\text{C}$ and $52 \pm 2\%$, respectively.

The seven monkeys were tested simultaneously in their home cages using a computer-automated training and testing system which measures and categorizes the percent correct at each delay, and the response latency at each step of the procedure. The computer and operator were isolated from the subjects. Each

experimental set-up consisted of a touch-sensitive screen and a pellet dispenser unit mounted in a light-weight aluminium chasis that could be attached to the home cage. A trial began by the presentation of a 5.7 (h) x 8.8 (w) cm coloured rectangle located in the upper center of the screen. Monkeys were trained to touch the illuminated sample (red, blue, or yellow) area to initiate a trial. This action also extinguished the sample. Following the computer-specified delay interval, two coloured choice rectangles, but not the sample, were illuminated. One of the two choices matched the sample colour, while the other (incorrect) choice was one of the two remaining colours. Correct trials (matches) were rewarded by the delivery of a reinforcement food pellet. There was a constant inter-trial interval of 5 sec and daily sessions consisted of 96 trials. The various combinations of stimulus colour were arranged so that each appeared an equal number of times as a sample; each colour appeared an equal number of times as choice and each colour appeared an equal number of times in combination with each other colour. Likewise, when two colours appeared in combination, each colour was counterbalanced between left and right sides of the screen in a non-predictable pattern.

Finally, all stimulus counterbalancing procedures were matched to length of delay interval. Monkeys exhibit individual capabilities to maintain matching performance following various delay intervals, and the longest delay chosen for a particular monkey was that which consistently allowed correct matching at just above chance levels (approximately 60% correct). In general, the length of delay interval was adjusted for each subject until 4 levels of performance difficulty were found: the least difficult zero delay (85-100% correct); a short delay (75-84% correct); a medium delay (65-74% correct) and a long delay (55-64% correct) representing each animal's limit of DMTS performance. Zero delay was included as a control to monitor for changes in reference memory and/or other potential non-mnemonic changes in task performance. Baseline data were obtained following the administration of drug vehicle.

> For Example 22, the individual best dose identified during the dose-range study 60 min after dosing was 0.3 mg/kg for 5 monkeys, 0.1 and 1 mg/kg for the 2 remaining monkeys. The results obtained 1h and 24 h after Example 22 administration were compared for the different delays. The analysis indicated that significant performance improvement was observed at long delay 1h after Example 22 best dose

administration ($P < 0.01$). Results indicate that Example 22 significantly enhanced DMTS performance in aged monkeys at the active dose of 0.3 mg/kg. The improvement was seen at long delay intervals suggesting a selective effect of Example 22 on memory or recall.

In conclusion, compound of the present invention possess cognitive enhancing properties. Neurochemical studies suggest that the cognition enhancing properties of compounds could be mediated not only by histaminergic neurotransmission facilitation, but also by facilitation of cholinergic or monoaminergic neurotransmission via pre-synaptic H_1 heteroreceptors located on cholinergic terminals.

The cognitive profile of the compounds suggests that they could counteract the episodic memory and working memory deficits which occurs during aging and early in Alzheimer's disease. Compounds improves memory retention in social recognition test and in object recognition test in rats (models of episodic memory) and in two other working memory tests: spontaneous alternation in the mouse and delayed Matching to sample task in aged monkeys. These results are in favour of a promising therapeutic potential of compounds of the present art for age-related cognitive and attentional disorders which occur in diseases such as Alzheimer's disease, Parkinson's disease, vascular dementias, ADHD, and mood disorders.

3- EXPERIMENTS CONCERNING MOOD DISORDERS

Concerning mood disorders the compounds of the present art have been tested in Tail Suspension Test in NMRI mice. Tail Suspension test permits to detect potential effects of compounds on mood disorders. Mice were caught by the tail using a piece of tape and suspended to a hook during 6 min. The occurrence of escape-related behaviour was recorded using an automated system (Bioseb, France). This apparatus has three readouts : the immobility (s) of the mouse, energy and power of movements. Mice were treated with Example 22 (5-10-20 mg/kg i.p.) or vehicle and isolated 30 min before the test.

> Example 22 increased the time spent in immobility during the suspension in a dose dependent manner with a significant effect for all tested doses without any effect on

energy and power of the movements. Results indicate that Example 22 could act as an atypical anxiolytic drug.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of the title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Further declarant sayeth not



Pierre LESTAGE

Executed at : Suresnes

Date : February, 23, 2009

Postal address

LES LABORATOIRES SERVIER

35, rue de Verdun

92284 Suresnes

FRANCE